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Study on Occurrence of Human Calicivirus (Mexico Strain) as Cause of Sporadic Cases and Outbreaks of Calicivirus-Associated Diarrhoea in the United Kingdom, 1983–1995

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The use of a recently developed EIA using antisera raised against purified baculovirus expressed recombinant Mexico virus (rMx) capsid protein is described for screening for human calicivirus in stools. The results show that MX-like viruses have been circulating in the UK periodically since 1983 and were an occasional cause of sporadic cases of diarrhoea in infants and outbreaks of infection among elderly patients in hospitals and old people's homes. Further evidence is presented that some strains of caliciviruses with characteristic surface morphology (HuCVs) and some with an indistinct appearance, small round structured viruses (SRSVs) are antigenically related to MxV. Tests on SRSVs from four unrelated outbreaks typed as UK3 failed to react in the Mx EIA or recombinant Norwalk virus (rNV) EIA.

A 2-month survey of 206 children treated in two London hospitals for diarrhoea showed that only one was positive for MxV, a child known to be infected with HIV-1. None of the samples reacted in the rNV EIA.

KEY WORDS: EIA, Mexico virus, small round structured virus, human calicivirus, Hawaii virus, Norwalk virus

INTRODUCTION

The success in sequencing several human caliciviruses [Jiang et al., 1993; Lambden et al., 1993] and the application of reverse transcription PCR (rt-PCR) using primers directed to the relatively conserved region of ORF1 encoding the RNA dependent RNA polymerase permitted the division of human caliciviruses into at least three genetically distinct groups. Group I includes viruses with a sequence similar to the prototype Norwalk virus, NV8FIIa [Jiang et al., 1993]—Southampton virus (SV) [Lambden et al., 1993] and Desert Shield virus (DSV) [Lew et al., 1994a]. Group II includes Snow Mountain agent (SMA)-like viruses:—Hawaii (HV) [Lew et al., 1994b], Toronto virus (TV) [Lew et al., 1994c], and Bristol virus (BV) [Green et al., 1994]—some strains of morphologically typical human calicivirus (HuCV) [Cubitt et al., 1994]. Group III includes morphologically typical strains of HuCV—Sapporo, Houston, Manchester, and Plymouth strains [Matson et al., 1995; Liu et al., 1995] which have a genomic organization that is distinct from Group I and II and closer to rabbit haemorrhagic disease virus. Recently a virus, Mexico virus (MxV), which falls within group II has been sequenced and the capsid protein expressed in baculovirus [Jiang et al., 1995b]. This has enabled the development of enzyme immune assays for detection of antigenically related viruses and allowed measurement of antibody responses. The results of the studies have shown that MxV-like viruses are present in Mexico and the United States [Jiang et al., 1995a]. The assay detects the prototype Hawaii and Snow Mountain agents and SRSVs and morphologically typical human caliciviruses that had been placed in genogroup II, based on 89–98% amino acid homology within the RNA polymerase region. In this study, only 1/24 samples that were positive in the MX EIA failed to be detected by rt-PCR using Norwalk primers 35,36. A seroepidemiological survey in London has shown that acquisition of antibodies to MxV occurs early in life and that the majority (>90%) of the population showed evidence of past infection with an antigenically related virus by the age of 4–6 years [Parker et al., 1995]. A similar pattern of acquisition was found in Mexico [Jiang et al., 1995b].

The present study was conducted to determine whether the MxV antigen EIA would be of diagnostic value for identifying infections with morphologically typical HuCVs and/or small round structured viruses (SRSV) by screening a collection of viruses that had

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been obtained between 1982–1995 from outbreaks and sporadic cases in the UK. All samples were also screened for the presence of NV.

MATERIALS AND METHODS

Sporadic Cases

Faecal samples were obtained from 75 children with symptoms of diarrhoea and/or vomiting who had been shown to be excreting SRSVs and from 28 children excreting morphologically typical HuCVs. These had been collected between 1982–1995 in north London.

A further 11 samples containing SRSVs were tested, four from children in Manchester collected in 1986–87, six sporadic cases from four children and two adults in Birmingham collected in the summer of 1994, and a single sample from a child in Spain collected in 1994. Sequence data on the polymerase region of ORF1 of three of the HuCVs and one of the SRSVs have been published previously [Cubitt et al., 1993].

Outbreaks

Faecal samples were still available from 183 adults involved in 38 outbreaks of diarrhoea and vomiting that occurred in the UK during the period 1986–95, (see Table II). A further nine specimens were obtained from patients from an outbreak that occurred in Turku, Finland, during 1988. Direct examination by electronmicroscopy (EM) of preparations stained negatively with 2% potassium phosphotungstic acid (KPTA), pH 6.4 had shown previously that 72/192 (38%) samples (see Table II) contained SRSVs. The SRSVs associated with four of the outbreaks had been typed by solid phase immune electronmicroscopy (SPIEM) and identified as stain "UK3" by Lewis [1991], Public Health Laboratory (Leeds, UK).

All samples had been stored either as stools at +4C in sealed containers or as ~10% faecal emulsions at −20C. Specimens that had become desiccated or obviously contaminated with fungi were not included in the study.

Survey of Children With Diarrhoea

Stool samples (260) were screened from children treated for diarrhoea at Great Ormond St Hospital for Children or Queen Elizabeth Hospital for Children during the period February–April 1995. Direct examination by electronmicroscopy of negatively stained samples had shown that 45 contained rotavirus, 5 SRSVs, 2 astrovirus, 2 adenovirus, and 1 morphologically typical HuCVs. A further sample was shown to contain poliovirus after cultivation in human embryonic lung fibroblasts.

Enzyme Immunosassay

Mx antigen assay. Alternate columns of 96-well microtitre plates (Immulon, Dynatech) were coated with 100 μl of 1:2,000 rabbit serum (collected before and after immunization with purified rMxV capsid protein) and held at 4°C overnight. The following day plates were washed in phosphate-buffered saline/tween (PBST) and blocked with 5% dried milk powder/PBS for 1 hour. After a further wash cycle, 100 μl of clarified faecal extract was add to wells coated with pre- and postchallenge sera and incubated for 2 hours at 37°C. Plates were washed five times in PBS/tween and 100 μl of a 1:2,000 dilution of hyperimmune guineapig anti-rMxV serum in 1% milk buffer added to each well. Plates were incubated for 1 hour at 37°C and then washed three times in PBS. To each well was added 100 μl of 1:8,000 goat antiguineapig IgG-horseradish peroxidase conjugate (Sigma, Poole, UK) and incubated at 37°C for 1 hour. Plates were washed three times in PBST and once in distilled water before adding tetramethyl benzidine substrate. The reaction was stopped after 20 minutes with sulphuric acid and the absorbance A_{450nm} read in an ELISA reader. Samples giving A_{450nm} of ≥ 0.1 and a Post/Pre (P/N) ratio of >2.0 were considered to be positive. Positive and negative stools were included on each plate as controls.

NV antigen assay. All samples were tested as described previously [Parker et al., 1993].

Detection of Viruses in Stool by rt-PCR

The CTAB method was used to extract RNA from stool extracts and rt-PCR performed using primers 35,36 as described previously [Jiang et al., 1992].

RESULTS

Tests on the 86 samples containing SRSVs showed that 17 (20%) samples obtained from children (Table I) reacted in the MxV EIA. One of these samples, 20476/91 (4S), had been sequenced previously and shown have an amino acid sequence in the *pol* region similar to SMA [Cubitt et al., 1994]. A further nine specimens were tested by rt-PCR using NV primers 35,36, and all produced positive results. The earliest of these specimens was collected in 1983. One of the four sporadic cases that occurred in Manchester was positive and a single sample obtained from a child in Spain. A_{450nm} values for positive samples ranged from 0.2–1.69 and P/N values from 2–11.2. None of the samples obtained from cases that occurred in Birmingham during 1994 reacted.

Of stools containing morphologically typical HuCVs, 8/28 (28.5%) gave positive reactions. The results of rt-PCR for three of these samples, (3C, 5C, and 12C) together with data on their sequence in the polymerase region of ORF1 have been published previously [Cubitt et al., 1994]. Two of the samples showed close homology with Mx-V, whereas 12C was closer to Hawaii virus. A further four samples gave a positive rt-PCR reaction using NV primers 35,36, but there was insufficient material to sequence them. One sample obtained from a child aged 8 months, which was repeatedly positive in the MX EIA failed to produce a product by rt-PCR. The majority (6/8) of the HuCVs that reacted in the MxV EIA were obtained between November 1991 and April 1992. The other two cases occurred in 1994. The 3D region of one other HuCV, which failed to react in the Mx or NV EIAs, has been sequenced and found to fall within the Group III viruses (Cubitt, Matson, Tamas, and Jiang, unpub. obs.).

Sporadic cases of Mx-like virus infection were identi-

TABLE I. MxV EIA Positive Cases*

Lab no.	Month/year	Age		Morphology	rt-PCR[a] NV35,36
3766	1983	Child	NA	SRSV	NT
16356	07/1983	15m	H	SRSV	+
13019	1984	11m	H	SRSV	NT
T	05/1987	Child	NA	SRSV	NT
P	05/1987	Child	NA	SRSV	NT
E467	04/1989	Child	H	SRSV	NT
9573	1989	2y	C	SRSV	+
20476(4S)	02/1990	5m	H	SRSV	+[b]
23681	10/1991	7m	N	SRSV	+
23889	11/1991	3w	N	SRSV	+
35312	09/1993	2m	C	SRSV	NT
35685	11/1993	7m	N	SRSV	+
35868	11/1993	13m	N	SRSV	+
35903	10/1993	14m	N	SRSV	NT
39979	06/1994	7y	N	SRSV	NT
40817	09/1994	22m	H	SRSV	+
46351	08/1995	6m	N	SRSV	+
Man/8679	1986	Child	H	SRSV	NT
NA/Spain	1994	Child	NA	SRSV	+
24339	12/1991	Child	NA	HuCV	NT
25266(5C)	01/1992	7m	C	HuCV	+[b]
25645	02/1992	5m	C	HuCV	+
25839	02/1992	4m	N	HuCV	+
25976(12C)	02/1992	11m	N	HuCV	+[b]
27092(3C)	04/1992	3w	C	HuCV	+[b]
38346	03/1994	16m	C	HuCV	+
38853	04/1994	8m	N	HuCV	neg

*Abbreviations: C = patients treated in casualty, advised, and discharged; H = hospitalised patient; N = nosocomial infection; NA = clinical data not available; NT = not tested.
[a]rt-PCR performed using Norwalk virus primers 35,36.
[b]Data available on the sequences and clinical history, Cubitt et al., 1993; Jiang et al., 1995a.

fied in 11 of the 13 years of the study period and showed no apparent seasonality. Data on the age of the children indicated that the vast majority were infected between the age of 5 and 18 months. Many of the children had been in the hospital for >3 days prior to the onset of symptoms, and therefore it seems probable that these infections were acquired in the hospital.

A comparison of the results of electronmicroscopy and the MxV EIA to test samples from adults involved in 39 outbreaks associated with SRSV infection are summarised in Table II. Samples obtained from patients involved in six outbreaks were found to be positive in the MxV EIA. All the cases were among elderly patients aged > 60 years. The majority of the outbreaks occurred in June–July 1986, one in July 1987 and the other in January 1989. Application of the Mx EIA to examine samples in these six outbreaks showed that it detected more positives (15) than EM, which detected nine. The use of the EIA identified all samples that were positive by EM. Four of the six outbreaks associated with Mx-like viruses occurred in units caring for the elderly where spread occurred by person-to-person transmission, and the other two were believed to have been due to consumption of contaminated food.

None of the 39 outbreaks were shown to be associated with NV infection. Samples obtained from the four episodes associated with the SRSV UK3 strain failed to show any significant reaction in the assay (A_{450nm} range 0–0.015).

The results of the survey conducted on samples collected between February and April 1995 showed that only 1/260 was positive. This sample was obtained from a child, aged 13 months, who was HIV-1 antibody positive. The five samples shown to contain SRSV and the one containing HuCV all gave negative results ODs < 0.05. There was no reaction in any of the samples containing rota-, astro-, adeno-, or polio viruses.

DISCUSSION

The results of the study of sporadic cases of morphologically typical HuCV and SRSV infections in children indicate that MxV-like viruses have been circulating in the community over most of the past 13 years. Alignment of the amino acid sequence in the *pol* region showed that three samples (4S, 3C, 5C) clustered with MxV in subgroup 2, whereas 12C was more closely aligned to HV and SMA in subgroup 1 of genogroup II [Jiang et al., 1995a], indicating that the MX assay will detect antigenically distinct strains within genogroup II. The observation of a case of MxV in Spain indicates the need for studies to be conducted in other countries worldwide. Further evidence is provided that some morphologically typical HuCVs and SRSVs are related to MxV, whereas others are antigenically distinct. Work is in progress to establish how many of the typical HuCVs and SRSVs that failed to react in the rMX EIA are related to HuCV/Sapporo and can be assigned to genogroup III.

TABLE II. Comparison of Electronmicroscopy and the MxV EIA for Study of Outbreaks of Diarrhoea and Vomiting Associated With SRSV Infection, 1987–1995*

Month/year	Location	Situation	EM	rMx EIA	Strain
01/86	Barnet	Geriatrics	3/6	0/6	$UK3_a$
06/86	Harrogate	OPH	1/8	4/8	
07/86	Eastbourne	Hotel	3/5	0/3	
07/86	Harrogate	OPH	1/4	3/4	
07/86	Ashford	Geriatrics	2/9	3/9	
07/86	Eastbourne	Hotel	2/3	2/3	
08/86	Bilton	Hotel	2/3	0/3	
05/87	Glasgow	Hospital	1/3	0/3	
06/87	Lincoln	Holiday camp	2/2	0/2	
07/87	Leicester	Geriatrics	1/1	0/1	
07/87	Brighton	Hotel	1/1	0/1	
08/87	Minehead	Geriatrics	5/5	0/5	$UK3_a$
08/87	Bridgewater	Hospital	2/2	0/2	$UK3_a$
09/87	Inverness	Hospital	3/3	0/3	
08/87	Reading	Hotel	2/6	2/6	
08/87	St Caths	Hotel	2/2	0/2	
1988	Oxford	Hospital	1/1	0/1	
1988	Wycombe	Hospital	1/1	0/1	
05/88	Epsom	Hotel	2/9	0/9	
06/88	Hereford	Holiday camp	2/13	0/13	
07/88	Eastbourne	Hotel	2/2	0/2	
09/88	Cardiff	OPH	4/5	0/5	
10/88	Reading	OPH	1/9	0/9	
1988	Birmingham	Geriatrics	2/5	0/5	
1988	Birmingham	Hospital	2/8	0/8	
1988	Brighton	Hotel	2/2	0/2	
10/88	Inverness	Hotel	3/4	0/4	
12/88	Finland	Hospital	4/9	0/9	$UK3_a$
01/89	Birmingham	Hospital	1/5	0/5	
1989	Birmingham	OPH	1/2	1/2	
1990	Kings Lynn	Geriatrics	3/3	0/3	
12/93	London	Hospital	3/3	0/3	
1994	Birmingham	Adults	1/1	0/1	
1994	Birmingham	Geriatrics	1/1	0/1	
1994	Birmingham	Geriatrics	1/1	0/1	
1994	Birmingham	Adults	2/2	0/2	
1994	Birmingham	Adults	1/1	0/1	
01/95	London	Geriatrics	4/39	0/39	
02/95	London	Geriatrics	2/5	0/5	

*Denominators reflect number of samples that were still available for testing from each outbreak; many EM negative samples had been discarded. Serotyping using SPIEM performed by D. Lewis, Public Health Laboratory, Leeds, UK. Samples from Birmingham courtesy of Dr. Desselberger and Dr. Beards. Samples from Turku, Finland supplied by Dr. O Meurmann.

The age distribution of cases indicates that infection with Mx-like viruses occurs commonly in the 12-month period 5–18 months after maternal antibody has disappeared. This correlates closely with the seroprevalence data in London [Parker et al., 1995] and contrasts with the pattern for Norwalk virus infection that appears to affect older children and adults [Parker et al., 1994]. Many of the cases were relatively mild and were treated in the Accident and Emergency Department with oral rehydration salts and the children discharged after being given appropriate advice. Others were detected because children developed diarrhoea while being treated in the hospital for other conditions.

The high prevalence of cases occurring while children were in the hospital suggests either that asymptomatic excretors may be common or the environment is heavily contaminated. Alternatively, HuCVs may have the potential to cause latent infections that reactivate when patients are immunedeficient or immunecompromised, a situation known to exist in cats infected with feline calicivirus [Studdert, 1978].

The results of the present study indicate that MxV-like viruses are a cause of outbreaks in the elderly either as a result of person to person transmission or the consumption of contaminated food. Interestingly, many of the outbreaks occurred in the summer months, particularly those that happened in hotels. A similar observation was made by of McDonell et al. [1995] in a survey of outbreaks in the UK.

Elderly people in closed communities are known to be highly susceptible to outbreaks of viral diarrhoea due to rotavirus [Cubitt et al., 1980], astrovirus [Lewis et al., 1989], and caliciviruses [Cubitt, 1989], probably due to decreased immunity and factors such as incontinence and poor hygiene. It is therefore not surprising that MxV outbreaks were identified in old people's homes and geriatric wards.

The majority, 4/6 outbreaks in 1986 were associated

with MxV-like viruses, 1/9 in 1987 and 0/12 in 1988, which suggests that different strains of calicivirus may be periodically circulating in the community similar to the situation recorded for rotaviruses [Noel et al., 1991]. Further support for this view comes from a survey of SRSV outbreaks in Yorkshire, UK, between 1990–92, which showed that the UK3 strain was the most prevalent strain in adults [Lewis et al., 1993]. The strains denoted as UK3 using the typing scheme developed by Lewis in 1991 have been reported to be antigenically related to Hawaii agent and fall within genogroup 2 [Ando et al., 1995]. However, the results of the present study show that none of the viruses obtained from outbreaks caused by UK3 reacted in the MxV EIA. Paired sera obtained from patients involved in three of the outbreaks showed no significance seroresponses in the rMX antibody EIA [Parker et al., 1995]. In contrast HV reacts in the rMX antigen EIA [Jiang et al., 1995a] and some volunteers challenged with HV showed low but significant seroresponses to rMxV [Jiang et al., 1995a]. The results indicate that the Mx EIA detected only one-fifth of the sporadic cases of calicivirus and none of the strains causing outbreaks in adults in recent years. There is therefore a need to continue to sequence and express other strains of calicivirus in order to develop diagnostic tests that will have a broader spectrum of activity.

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